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Title:

SYSTEMS AND METHODS FOR DETECTION OF LOW CONCENTRATIONS OF
MOLECULES USING SURFACE ENHANCED RAMAN SPECTROSCOPY

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SYSTEMS AND METHODS FOR DETECTION OF LOW CONCENTRATIONS OF MOLECULES USING SURFACE ENHANCED RAMAN SPECTROSCOPY

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a continuation-in-part of U.S. Patent Application Serial No. 09/852,992, entitled "SYSTEM AND METHOD FOR CONTROLLING DEPOSITION PARAMETERS IN PRODUCING A SURFACE TO TUNE THE SURFACE'S PLASMON RESONANCE WAVELENGTH," filed May 10, 2001, the entire disclosure of which is incorporated herein by reference.

TECHNICAL FIELD

[0002] The present invention is related in general to molecular analysis and more particularly to systems and methods for detection of low concentrations of molecules using surface enhanced Raman spectroscopy.

BACKGROUND OF THE INVENTION

[0003] Various techniques have been developed to analyze the structure of and/or identify molecular scale structures. Techniques that permit analysis and/or detection of single molecules include laser induced fluorescence, near field microscopy, scanning probe spectroscopy, magnetic resonance, and Raman spectroscopy. Laser induced fluorescence provides a relatively high signal to noise ratio to permit detection of the respective analyte molecule. However, laser induced fluorescence provides limited structural information. Moreover, laser induced fluorescence requires a specific fluorescent "tag" or "dye" molecule for a specific analyte molecule. Accordingly, laser induced fluorescence is not appropriate as a general analytic technique. Scanning probe spectroscopy provides atomic resolution and relatively high sensitivity. However, scanning probe spectroscopy provides limited absorbate structural information. Near field microscopy provides improved spatial resolution. However, near field microscopy is associated with relatively low optical throughput. Fluorescence detected magnetic resonance provides acceptable sensitivity. However, fluorescence detected magnetic resonance is associated with extremely limited molecular applicability.

[0004] Raman spectroscopy relies upon Raman scattering to analyze analyte molecules. Raman scattering is a process where photons change energy states when the photons interact with a respective molecule or material. The frequency shift may result from gaining energy from or losing energy to vibrational (e.g., phonons), rotational, or other energy states associated with the material. The frequency shift phenomenon was discovered in 1928 by C.V. Raman.

[0005] Raman discovered that when certain molecules are illuminated, a small percentage of the molecules, which interact with a respective photon, transition to a different vibrational or energy state than originally occupied before interaction with the photon. If a respective molecule transitions to a higher energy state than originally occupied before interaction with a photon, the photon is scattered and thereby possesses a lower energy state (i.e., a longer wavelength). This is commonly referred to as "Stokes-shifted Raman scattering." If a molecule transitions to a lower vibrational or energy state than originally occupied before interaction with a respective photon, the respective photon is scattered and thereby possesses a higher energy state (i.e., shorter wavelength). In this case, the process is commonly referred to as "anti-Stokes-shifted Raman scattering."

[0006] In a set of molecules under normal conditions, the number of molecules at ground state is generally much greater than those at an excited state. Therefore, the probability of an incident photon interacting with an excited molecule and being scattered with more energy is typically very small. Thus, when a set of molecules are under analysis, photon scattering at frequencies higher than that of the incident photons (anti-Stokes frequencies) is typically minor relative to that at frequencies lower than that of the incident photons (Stokes frequencies). Consequently, the Stokes frequencies are usually analyzed in Raman spectroscopy processes of the prior art.

[0007] The amount of energy lost to, or gained from, a molecule in this way is quantized, resulting in the scattered photons having discrete wavelength shifts. These wavelength shifts can be measured by a spectrometer. Raman scattering was initially considered to have the potential to be useful as an analytical tool to identify certain molecules, and as a means of studying molecular structure. Specifically, the wavelength distribution of the Raman spectra may be analyzed to identify energy levels associated with the material and the distribution of occupied states in the energy levels. The information obtained in this manner may

facilitate an understanding of the molecular geometries, chemical bonding, and interaction of molecules in solution associated with the material, as examples. However, interest in Raman scattering initially faded somewhat as other methods, such as infrared spectroscopy, gained popularity.

[0008] Interest in Raman spectroscopy was renewed with the advent of the laser as a light source. Its intense coherent light overcame some of the sensitivity drawbacks initially encountered in Raman spectroscopy. Moreover, it was discovered that when the wavelength of the incident light is at or near the maximum absorption frequency of the molecule, and hence causes coupling with electronic energy states as well as vibrational transitions in the molecules, resonance Raman scattering is observed. With resonance Raman scattering, the scattered photons still show the differences in vibrational energy. However, with resonance Raman scattering, the absorption is approximately 1000 times more efficient. Even with the increased signal from resonance Raman scattering, its usefulness as an analytic tool was limited due to its still comparatively weak signal.

[0009] Interest in Raman spectroscopy further increased when, in 1974, M. Fleischmann et al. discovered surface-enhanced Raman spectroscopy (SERS), although it was not immediately recognized as such. See M. Fleischmann, P.J. Hendra, and A.J. McQuillan, Chem. Phys. Lett., 1974, 26, 163. Specifically, Fleischmann et al. observed intense Raman scattering from pyridine adsorbed onto a roughened silver electrode surface from aqueous solution. Fleischmann's approach was to roughen the electrode to increase its surface area and, hence, the number of adsorbed molecules available for study. Dr. Richard P. Van Duyne et al. later recognized that the large intensities observed could not be accounted for simply by the increase in the number of scatterers present and proposed that an enhancement of the scattered intensity occurred in the adsorbed state. See D.L. Jeanmaire and R.P. Van Duyne, J. Electroanalytic Chem., 1977, 84, 1.

[0010] Also in 1977, Creighton et al. recognized that the increased Raman signal was not possible by more scatterers alone and proposed an enhancement mechanism. See M.G. Albrecht and J.A. Creighton, J. Am. Chem. Soc. 5215 (1977). Thus, it was recognized that Raman scattering efficiency can be enhanced when a compound is adsorbed on or near special metal surfaces suspended in appropriate solutions. That is, a significant increase in the intensity

of Raman light scattering can be observed when molecules are brought into close proximity to (but not necessarily in contact with) certain metal surfaces that are atomically "roughened." Metal colloids also demonstrate such signal enhancement effect. Such phenomenon is referred to as "surface-enhanced Raman scattering" (SERS), and use of such surface-enhancement has enabled enhancements in Raman scattering efficiency by factors of 10^6 to be observed.

[0011] The cause of the SERS effect is not completely understood. However, at least two separate factors contributing to SERS have been advanced in the prior art. First, the metal surface contains minute irregularities. These irregularities may be thought of as spheres (in a colloid, they are spheroidal or nearly so). Those particles with diameters of approximately 1/10th the wavelength of the incident light have been thought to contribute most to the effect. The incident photons induce a field across the particles which, being metal, have very mobile electrons.

[0012] In certain configurations of metal surfaces or particles, groups of surface electrons can be made to oscillate in a collective fashion in response to an applied oscillating electromagnetic field. Such a group of collectively oscillating electrons is called a "plasmon." The incident photons supply this oscillating electromagnetic field. The induction of an oscillating dipole moment in a molecule by incident light is the source of the Raman scattering. The effect of the resonant oscillation of the surface plasmons is to cause a large increase in the electromagnetic field strength in the vicinity of the metal surface. This results in an enhancement of the oscillating dipole induced in the scattering molecule and hence increases the intensity of the Raman scattered light. The effect is to increase the apparent intensity of the incident light in the vicinity of the particles.

[0013] A second factor considered to contribute to the SERS effect is molecular imaging. A molecule with a dipole moment, which is in close proximity to a metallic surface, will induce an image of itself on that surface of opposite polarity (i.e., a "shadow" dipole on the plasmon). The proximity of that image is thought to enhance the power of the molecules to scatter light. In other words, this coupling of a molecule having an induced or distorted dipole moment to the surface plasmons greatly enhances the excitation probability. The result is a very large increase in the efficiency of Raman light scattered by the surface-absorbed molecules.

[0014] The SERS effect can be enhanced through combination with the resonance Raman effect. When an excitation light source (e.g., laser) used to excite SERS is in resonance with an electron state transition of the substance, such condition is referred to as surface-enhanced resonance Raman scattering (or "SERRS" or "resonant SERS"). As described above, an enhancement in the efficiency of Raman scattering on the order of 10^6 fold has been observed with SERS. An additional 10^3 fold enhancement in the efficiency of Raman scattering has been observed with SERRS.

[0015] Accordingly, surface-enhanced Raman spectroscopy (both SERS and SERRS) are capable of providing information for use in identifying and analyzing molecules. However, known techniques that utilize SERS and SERRS are problematic. First, known SERS and SERRS techniques are associated with substrate reliability issues.

BRIEF SUMMARY OF THE INVENTION

[0016] The present invention is directed to a system and method which analyze molecules utilizing surface enhanced resonance Raman spectroscopy. In embodiments of the present invention, the absorption maximum of an analyte molecule is preferably determined. The absorption maximum of the analyte molecule may be determined through known techniques. For example, light at various wavelengths may be applied to a number of the analyte molecules. Transmitted and/or reflected light may be measured. The wavelength of light that minimizes the energy associated with transmitted and/or reflected light is associated with the absorption maximum. Embodiments of the present invention further tune a laser source to the absorption maximum wavelength. The laser source may be tuned by utilizing any number of techniques such as utilizing a suitable fiber Bragg grating feedback arrangement which is known in the art. Additionally, the Raman scattered wavelengths associated with the analyte molecule may be determined by applying optical energy from the tuned laser source to a relatively large sample of analyte molecules.

[0017] Embodiments of the present invention may advantageously tune the surface plasmon resonance wavelength (SPRW) to the wavelength associated with the absorption maximum of the analyte molecule. Embodiments of the present invention enable such tuning of the SPRW of the substrate by controlling a deposition process (e.g., a thermal evaporation process, sputter deposition, or chemical vapor deposition) utilized to create the substrate. For instance, a particular deposition process may be analyzed by varying at least one deposition parameter for respective substrate. Thus, various sample substrates may be obtained with each sample substrate produced according to the respective deposition parameter value(s). The sample substrates obtained may then be analyzed to determine the resulting SPRW for each sample substrate. The sample substrate that produces the largest surface-enhanced spectroscopy enhancement may be utilized as the selected substrate for a suitable detection system. In general, the sample substrate that will produce the greatest amount of surface-enhanced spectroscopy enhancement will be associated with the greatest overlap between the SPRW spectrum, the excitation spectrum, and the Raman scattered wavelengths (as identified by the Stokes-shift spectrum). The sample substrate that produces the largest surface-enhanced spectroscopy enhancement may be determined utilizing either empirical or computational methods.

[0018] Moreover, embodiments of the present invention may utilize other techniques to increase the sensitivity of surface-enhanced Raman spectroscopy. For example, embodiments of the present invention may utilize metal particle gap enhancement, chemical enhancement, and/or microcavity enhancement techniques. By tuning the plasmon resonance wavelength of a suitable surface and by utilizing one or more other enhancement techniques, embodiments of the present invention enable detection of exceptionally small numbers of particles and, potentially, even enable detection of a single molecule.

[0019] The foregoing has outlined rather broadly the features and technical advantages of the present invention in order that the detailed description of the invention that follows may be better understood. Additional features and advantages of the invention will be described hereinafter which form the subject of the claims of the invention. It should be appreciated by those skilled in the art that the conception and specific embodiment disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present invention. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope of the invention as set forth in the appended claims. The novel features which are believed to be characteristic of the invention, both as to its organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] For a more complete understanding of the present invention, reference is now made to the following descriptions taken in conjunction with the accompanying drawing, in which:

[0021] FIGURE 1 depicts a table of deposition parameters for tunable substrates and the resulting surface resonance plasmon wavelength according to embodiments of the present invention;

[0022] FIGURE 2 depicts extinction spectra for the substrates listed in FIGURE 1 according to embodiments of the present invention;

[0023] FIGURES 3A and 3B depict the predicted and measured surface plasmon wavelengths and their differences for the substrates listed in FIGURE 1, and illustrate that the SPRW decreases monotonically with substrate temperature and increases monotonically with film thickness;

[0024] FIGURES 4A-4C depicts images of respective tuned surfaces captured utilizing atomic force microscopy according to embodiments of the present invention;

[0025] FIGURE 5A depicts measured Raman spectra using 514.5 nm excitation radiation collected at four positions on a tunable substrate according to embodiments of the present invention.

[0026] FIGURE 5B depicts the low intensity measured Raman spectra depicted in FIGURE 5A with the plot re-scaled to provide greater detail;

[0027] FIGURE 6A depicts measured Raman spectra using 606.3 nm excitation radiation collected on a plurality of tunable substrates according to embodiments of the present invention;

[0028] FIGURE 6B depicts the peak Raman scattered spectra measured according to the empirical trials for a plurality of SPRW values;

[0029] FIGURE 7 depicts a flowchart of steps associated with surface enhance resonance Raman spectroscopy according to embodiments of the present invention;

[0030] FIGURE 8 depicts another flowchart of steps associated with surface enhance resonance Raman spectroscopy according to embodiments of the present invention; and

[0031] FIGURE 9 depicts a system for detecting very small concentrations of analyte molecules utilizing surface enhance resonance Raman spectroscopy according to embodiments of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0032] Embodiments of the present invention use tunable surface materials as described in U.S. Patent Application Serial No. 09/852,992, entitled "SYSTEM AND METHOD FOR CONTROLLING DEPOSITION PARAMETERS IN PRODUCING A SURFACE TO TUNE THE SURFACE'S PLASMON RESONANCE WAVELENGTH," and "Tunable surface plasmon resonance silver films," *Applied Physics Letters*, Vol. 79, Number 19, pp. 3164-3166 (5 November 2001) by W.A. Weimer and M.J. Dyer, which are incorporated herein by reference to enable detection of molecules. Embodiments of the present invention enable the surface plasmon resonance wavelength (SPRW) of the substrate to be tailored to a desired analyte molecule.

[0033] As described in the identified patent application and the article by W.A. Weimer and M.J. Dyer, a suitable surface material may be produced by depositing a film on a substrate. In embodiments of the present invention, a thermal evaporator deposition technique is utilized to tune the film to a desired SPRW. The deposition technique may, for example, deposit a film comprising a suitable metallic material (e.g., silver (Ag), gold (Au), copper (Cu), and/or the like) onto the substrate. The substrate may be implemented utilizing glass, metal, or suitable dielectric materials as examples. The deposition technique may be advantageously optimized by selecting the substrate temperature, deposition rate, and film thickness to achieve a desired SPRW. In embodiments of the present invention, the ranges for the substrate temperature, deposition rate, and film thickness are 31 °C to 120 °C, 0.3 Å/s to 1.2 Å/s, and 30 Å to 120 Å, respectively. It shall be appreciated that the ranges are exemplary and other values may be utilized according to embodiments of the present invention.

[0034] Using a three-factor, three-level Box-Behnken experimental design, experimental results may be obtained. The experimental results may determine the maximum extinction wavelength for a given set of deposition parameters. FIGURE 1 depicts experimental results 100 (including SPRW) associated with tunable films for respective deposition parameter combinations. Also, FIGURE 2 depicts extinction graph 200 that illustrates the extinction spectra associated with the tunable film surfaces of the substrates shown in table 100 of FIGURE 1. It shall be appreciated that the SPRW for a tunable substrate corresponds to the wavelength associated with the maximum extinction. Also, an examination of the range of SPRWs

demonstrates that the SPRW may be varied from 540 nm through higher wavelengths in the visible and into the near-infrared regions of the electromagnetic spectrum according to embodiments of the present invention.

[0035] In accordance with embodiments of the present invention, the SPRW of deposited tunable surface plasmon resonance film may be calculated by the following formula:

$$\lambda_{PW} = 553 - 0.689T_s + 2.79R_d + 5.16T_f + 0.00503T_s^2 - 0.262T_sR_d - 0.0185T_sT_f + 17.1R_d^2 - 0.0R_dT_f - 0.0245T_f^2$$

where λ_{PW} is the surface plasmon resonance wavelength in nm, T_s is the substrate temperature in °C, R_d is the deposition rate in Å/s, and T_f is the film thickness in Å.

[0036] FIGURE 3A depicts a graph 300, in which the predicted surface plasmon wavelengths for the substrates listed in FIGURE 1 are illustrated by line 310. The difference between the predicted surface plasmon wavelengths illustrated by line 310 and the measured surface plasmon wavelengths for the substrates listed in FIGURE 1 is illustrated by line 320. As indicated by line 320, there is substantial agreement between the predicted and measured surface plasmon wavelengths. In FIGURE 3B, graph 350 illustrates that the SPRW decreases monotonically with substrate temperature and increases monotonically with film thickness. Accordingly, any suitable SPRW within the identified spectrum may be obtained by varying the respective parameters according to embodiments of the present invention.

[0037] FIGURES 4A-4C respectively depict images 401-403 of the surfaces of substrates 11, 14, and 12 created via deposition utilizing the parameter combinations shown in FIGURE 1. Images 401-403 of FIGURES 4A-4C were captured utilizing tapping mode atomic force microscopy (AFM). As shown in FIGURE 1, substrates 11, 14, and 12 are associated with SPRWs respectively of 552 nm, 602 nm, and 650 nm. As shown in FIGURES 4A-4C, the SPRW increases in accordance with the size of the particles deposited on the substrate.

[0038] FIGURE 5A depicts graph 500 of measured Raman spectra using 514.5 nm excitation radiation collected at four positions 508, 510, 512 and 514 on a tunable substrate 502 according to embodiments of the present invention. Tunable substrate 502 comprises a glass

substrate with a film 504 and a sample 506 deposited thereon. The film 504 comprises a gold film. The sample 506 comprises a 5.0 μL of rhodamine 6G (R6G) dye/methanol solution that was drop-casted onto the gold film 504 deposited on the glass substrate 502 with heating, which caused the solution to evaporate and leave behind an R6G sample 506 on the film 504. Position 508 is on the sample 506, but off the film 504. Position 510 is on the sample 506 and on the film 504. Position 512 is off the sample 506 and on the film 504. Position 514 is off the sample 506 and of the film 504. FIGURE 5B depicts graph 550 of the low intensity measured Raman spectra depicted in FIGURE 5A with the plot re-scaled to provide greater detail. In graph 500, it is seen that the spectra are dominated by the fluorescence exhibited at position 508, which is on the sample 506 and off the film 504. As expected, no signal is observed at positions 512 and 514, both of which are off the sample 506. However, an appreciable SERS signal is observed for the spectrum collected at position 510, which is on the sample 506 and on the film 504.

[0039] FIGURE 6A depicts measured Raman spectra using 606.3 nm excitation radiation collected on a plurality of tunable substrates according to embodiments of the present invention that are illustrated by FIGURE 1. In particular, FIGURE 6A depicts Raman spectra using 606.3 nm excitation radiation for substrates 12, 7, 10, 14 and 2 illustrated in FIGURE 1. It is assumed that essentially all of the SERS signal measured is enhanced by the gold film. The SERS enhancement depends upon matching both the excitation wavelength and the wavelength of the Raman scattered light. Examination of extinction graph 200 of FIGURE 2 reveals that the extinction spectra are actually quite broad, and it is concluded that the enhancement may be maximized when the greatest overlap between the surface plasmon resonance wavelength spectrum, the excitation wavelength, and the Raman scattered wavelengths exist.

[0040] In empirical trials, tunable gold substrates deposited according to embodiments of the present invention have demonstrated the highest SERS enhancement factors for tunable dry substrates to date. In the empirical trials, gold shot (ESPI, 99.999%) was deposited onto 17.00 mm diameter borosilicate glass coversheets utilizing thermal evaporation to form 11.4 mm diameter gold films according to embodiments of the present invention. The gold coated glass substrates were heated. Then, 5.0 μL of rhodamine 6G (R6G) dye/methanol solution was drop-casted onto the heat gold coated glass substrates thereby causing the solution to evaporate and leaving behind a R6G film. The R6G film possessed approximately 3.3×10^{13} molecules/ cm^2 .

Thus, each R6G molecule occupies 300 \AA^2 . Using a semi-empirical molecular orbital model (AM1) for the optimized structure of R6G, the maximum surface area per molecule on the surface is calculated to be 222 \AA^2 resulting in coverage that is roughly 74% less than a monolayer. Excitation of the R6G film deposited on the gold surface was performed using 514.5 nm radiation at 75 \mu m or 606.3 nm radiation at 70 \mu m to ensure nonresonant Raman excitation. The Raman scattered spectra was determined.

[0041] FIGURE 6B depicts Raman scattered peak spectra 650 measured according to the empirical trials for a plurality of SPRW values. The largest peak height in Figure 6B is about 1 cps, which is roughly 3 orders of magnitude less than the fluorescence signal of R6G at the same wavelength. Thus, the cross section for this nonresonant SERS signal is about 10^{-19} , corresponding to an enhancement factor for the gold film of about 10^{11} . This represents the highest known nonresonant SERS enhancement factor reported to date for a tunable dry film. Note that this enhancement factor assumes that all 2.0×10^5 R6G molecules in the probed area contribute to the signal equally. However, single molecule SERS analyses attribute strong SERS signals from relatively few ($\sim 0.1\%$) molecules residing on so called "hot spots." If only 0.1% of the molecules samples in the current work contribute to the measured signal, then the enhancement factor for those sites would be 10^3 higher, or 10^{14} , which is on the same order as the highest SERS enhancement factors reported to date for any system.

[0042] Additionally, tunable substrates according to embodiments of the present invention provide advantages other than improved SERS enhancement. Specifically, since tunable substrates are formed utilizing thermal deposition according to embodiments of the present invention, deposition may occur on irregular glass or, potentially, any stable dielectric surface. Thus, tunable substrates may be utilized in a wide variety of small volume applications. For example, tunable substrates may be used in applications that involve capillaries and microfluidic channels to facilitate ultrasensitive SERS detection.

[0043] FIGURE 7 depicts exemplary flowchart 700 according to embodiments of the present invention. Flowchart 700 begins at step 701 where the wavelength associated with the maximum absorption of an analyte molecule is determined. In step 702, a suitable laser may be tuned to the wavelength determined in step 701. In step 703, the SPRW of a suitable substrate is tuned to the wavelength determined in step 701 according to the controlled deposition techniques

of embodiments of the present invention. By tuning the laser and the substrate SPRW to the determined wavelength, embodiments of the present invention enable appreciable Raman enhancement to occur. Moreover, the Raman enhancement may occur in a suitable environment. Specifically, the Raman analysis may occur on a "dry surface" (i.e., on the tunable substrate) as opposed to utilizing a colloid solution. It shall be appreciated that the present invention is not limited to only tuning the laser and the SPRW to the determined wavelength. Additional Raman enhancement techniques may be employed as will be discussed in greater detail below.

[0044] In step 704, a sample to be analyzed is illuminated on the tuned substrate by the tuned laser. In step 705, the Raman scattered spectrum from the illumination is analyzed according to Raman spectroscopy techniques that are known in the art. In step 706, a logical comparison is made to determine whether the analyzed Raman scattered spectrum is consistent with the Raman scattered spectrum of the analyte molecule. If the logical comparison is true, the process flow proceeds to step 707 where detection of the analyte molecule may be communicated to a user. If the logical comparison of step 707 is false, the process flow proceeds to step 708 where it may be communicated that the analyte molecule is not present.

[0045] FIGURE 8 depicts exemplary flowchart 800 according to embodiments of the present invention. Flowchart 800 begins at step 801 wherein the wavelength associated with the maximum absorption of an analyte molecule is determined. In step 802, a laser is tuned to the wavelength determined in step 801. In step 803, the Raman scattered spectrum produced by the analyte molecule when illuminated by the tuned laser is determined. In step 804, a substrate is selected from a plurality of substrates that is associated with a largest overlap between the SPRW spectrum of the substrate, excitation wavelength, and Raman scattered wavelengths. The substrate associated with the largest overlap may be determined by illuminating a sample of the analyte molecule on each substrate of the plurality of substrates. The substrate that is associated with a largest peak intensity in the Raman scattered spectrum is the substrate that possesses the largest overlap.

[0046] In step 805, a sample to be analyzed is illuminated on the selected substrate utilizing the tuned laser. By tuning the laser and by selecting the appropriate substrate, embodiments of the present invention enable appreciable Raman enhancement to occur. It shall be appreciated that the present invention is not limited to only tuning the laser and selecting the

appropriate substrate. Additional Raman enhancement techniques may be employed as will be discussed in greater detail below. In step 806, the Raman scattered spectrum produced by illumination of the sample on the selected substrate is analyzed. In step 807, a logical comparison is made to determine whether the analyzed Raman spectrum is consistent with the Raman scattered spectrum of the analyte molecule. If the logical comparison of step 807 is true, the process flow proceeds to step 808 where it is communicated that the analyte molecule has been detected in the sample. If the logical comparison of step 808 is false, the process flow proceeds to step 809 where it is communicated that the analyte molecule is not present in the sample.

[0047] Embodiments of the present invention may utilize other techniques to achieve greater Raman enhancement. For example, embodiments of the present invention may utilize microcavities doped with nanostructured fractal aggregates which are described in "Fractals in Microcavities: Giant Coupled, Multiplicative Enhancement of Optical Responses," *Phys. Rev. Lett.* Vol. 82, Issue 24, pp. 4811-4814 (1999), by W. Kim, V.P. Safonov, V.M. Shalaev, and R.L. Armstrong, which is incorporated herein by reference. The microcavities described by Kim et al. are associated with fractal aggregate optical excitations concentrated in regions smaller than the diffraction limit of convention optics resulting in large local fields. Seeding the aggregates in the microcavities further increases the local fields due to light trapping by microcavity resonance modes. Accordingly, embodiments of the present invention may enclose a tuned substrate according to embodiments of the present invention in a microcavity to achieve a greater SERS enhancement factor.

[0048] Embodiments of the present invention may further optimize the metal particle gap. As previously noted, the tunable substrates according to embodiments of the present invention possess surfaces with varying particle size (see, for example, FIGURES 4A-4C). Optimizing the shape of the gap of the surface in which the analyte molecule may sit provides an additional enhancement to the Raman scattering.

[0049] Embodiments of the present invention may further provide chemical enhancement. Chemical enhancement involves causing the analyte molecule to chemically bind to the substrate surface. As is known in the art, chemical enhancement involves changes to the absorbate (film) electronic states due to chemisorption of the analyte.

[0050] As is known in the art, the typical cross-sections for the purposes of Raman scattering by potential analyte molecules are on the order to $10^{-30} \text{ cm}^2 \text{ molecule}^{-1}$. In light of the typical cross-sections of potential analyte molecules, an SERS enhancement factor of 10^{14} may provide the possibility of detecting a single molecule. Specifically, such an SERS enhancement factor may cause the Raman scattered signal from a single analyte molecule to be sufficiently strong to permit detection by known opto-electronic equipment.

[0051] Moreover, it is estimated that matching the SPRW of the tunable substrate according to embodiments of the present invention with the excitation and scattering wavelengths may provide an enhancement factor from 10^3 to 10^6 . Tuning the excitation (e.g., the laser source) to the wavelength associated with the maximum extinction of the analyte molecule may provide an enhancement factor from 10^3 to 10^5 . Optimizing the metal particle gap may provide an enhancement factor from 10^3 to 10^6 . Chemical enhancement of the substrate may provide an enhancement factor from 10^1 to 10^2 . Additionally, microcavity enhancement may provide an enhancement factor from 10^3 to 10^5 .

[0052] By utilizing tunable substrates according to embodiments of the present invention and by employing other known SERRS enhancement techniques, embodiments of the present invention enable attainment of the desired SERRS enhancement factor of 10^{14} or greater. Accordingly, it is believed that embodiments of the present invention are capable of detecting very small quantities of an analyte molecule. Specifically, embodiments of the present invention enable detection of sub-part per trillion levels of chemical analytes using surface enhanced Raman spectroscopy. Moreover, theoretical predictions suggest that embodiments of the present invention may provide sufficient SERRS enhancement to permit detection of a single molecule.

[0053] FIGURE 9 depicts detection system 900 according to embodiments of the present invention. System 900 comprises laser 901. Laser 901 is preferably tuned to a particular wavelength or wavelengths that are associated with an analyte molecule to be detected. If a sufficient enhancement factor is provided, a lower power, non-coherent light source may be utilized in lieu of laser 901 (e.g., a light emitting diode). System 900 further comprises focusing optics 902 to focus the output beam from laser 901 onto a portion of coated capillary 903. Coated capillary 903 may advantageously possess a surface deposited according to embodiments of the present invention. The deposition may cause the surface of coated capillary 903 to possess

a desired SPRW. Moreover, coated capillary 903 may be modified to optimize the metal particle gap and to provide chemical enhancement for the particular analyte molecule. Coated capillary 903 may also possess microcavities to provide additional Raman enhancement via the resonance modes of the microcavities.

[0054] System 900 further comprises dispersive optics 904 (e.g., a prism, a grating, an acousto-optic transducer, and/or the like). Dispersive optics 904 are preferably operable to angularly separate the respective wavelength components of the light scattered by the surface of coated capillary 903. The dispersed light is detected by optical detectors 905-1 through 905-N. Optical detectors 905-1 through 905-N may be implemented utilizing any number of suitable structures including photodiodes, a photodiode array, a charge-coupled device (CCD) array, and/or photomultiplier tubes. Optical detectors 905-1 through 905-N determine an amount of optical energy or power at respective wavelengths due to their respective positions relative to dispersive optics 904.

[0055] Controller 906 is preferably communicatively coupled to optical detectors 905-1 through 905-N via, for example, a suitable system bus (not shown). Controller 906 may comprise processor 910 that operates under the control of control logic 911. Control logic 911 may be implemented utilizing, for example, suitable executable instructions stored on a computer readable memory (e.g., magnetic medium, optical medium, PROM, EPROM, EEPROM, and/or the like). Control logic 911 may comprise executable instructions to analyze the Raman spectrum as detected by optical detectors 905-1 through 905-N to determine whether the detected spectrum is consistent with the analyte molecule. The executable instructions defining the Raman analysis may be implemented according to Raman analysis techniques that are known in the art.

[0056] In embodiments of the present invention, control logic 911 of controller 906 may be implemented to enable controller 906 to communicate information representative of the detected spectrum to another system for analysis. For example, controller 906 may be communicatively coupled to communication network 908 (e.g., the Internet). Controller 906 may communicate the detected spectrum information to remote monitoring system 909 via communication network 908. Remote monitoring system 909 may comprise processor 910 and control logic 911 to analyze the received information.

[0057] System 900 may be utilized for any number of applications. System 900 may be utilized as a general purpose analytic system. System 900 may, for example, facilitate nanofabrication or nanosynthesis processes. System 900 may be utilized to monitor ambient air quality conditions near a manufacturing facility. Additionally, system 900 may be implemented in a portable form to communicate to remote monitoring system 909 via a wireless communication network (e.g., by satellite). In this configuration, the detection elements (laser 901, focusing optics 902, coated capillary 903, dispersive optics 904, optical detectors 905-1 through 905-N, and controller 906) may be implemented as a device worn by military personnel in a hostile environment. In this environment, system 900 may be tuned to detect chemical or biological warfare agents. It shall be appreciated that these applications are merely exemplary. Embodiments of the present invention may be utilized for any number of applications that would benefit from the high sensitivity of enhanced surface-enhanced Raman spectroscopy.

[0058] Embodiments of the present invention may provide several advantages over known analytical techniques. Embodiments of the present invention enable detection of very low concentrations of molecules (e.g., sub-part per trillion or potentially single molecule detection). Additionally, embodiments of the present invention enable information regarding the molecular structure of the analyzed molecule to be captured. Specifically, as is known in the art, the Raman scattered spectrum is associated with the molecular geometries and the chemical bonds of analyzed molecules. Accordingly, embodiments of the present invention enable identification of analyzed molecules at very low concentrations, as opposed to merely detecting their structure. Moreover, embodiments of the present invention may operate in real-time. Specifically, embodiments of the present invention do not require preconcentration of the analyte and embodiments of the present invention may generate and analyze the Raman signals in seconds.

[0059] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined by the appended claims. Moreover, the scope of the present application is not intended to be limited to the particular embodiments of the process, machine, manufacture, composition of matter, means, methods and steps described in the specification. As one of ordinary skill in the art will readily appreciate from the disclosure of the present invention, processes, machines, manufacture, compositions of

matter, means, methods, or steps, presently existing or later to be developed that perform substantially the same function or achieve substantially the same result as the corresponding embodiments described herein may be utilized according to the present invention. Accordingly, the appended claims are intended to include within their scope such processes, machines, manufacture, compositions of matter, means, methods, or steps.